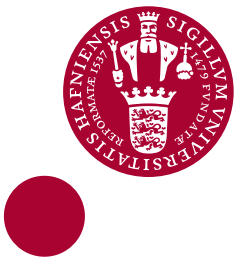


The exercise timing hypothesis: Can exercise training compensate for the reduction in blood vessel function after menopause if timed right?

Gliemann, Lasse; Hellsten, Ylva

Published in:
Journal of Physiology

DOI:
10.1113/JP277056

Publication date:
2019

Document version
Peer reviewed version

The exercise timing hypothesis - Can exercise training compensate for the reduction in blood vessel function after menopause if timed right?

L. Gliemann and Y. Hellsten, *Department of Nutrition, Exercise and Sports, University of Copenhagen, Denmark*

Wordcount: 4311

Abstract

As women enter menopause at mid-life, estrogen production ceases and its many beneficial effects on cardiovascular health are lost whereby the age-related risk of cardiovascular disease is accelerated. Estrogen acts via estrogen receptors and can activate the estrogen response element leading to upregulation of a number of proteins of importance for vascular health, including the vasodilator and anti-atherogenic enzyme endothelial nitric oxide synthase and angiogenic factors. Hormone replacement therapy can to some extent counteract the loss of estrogen although studies have shown that such treatment may only be effective if initiated soon after menopause, the so-called timing hypothesis. An attractive alternative to hormone therapy is regular physical activity, as it is known that exercise induces many of the same cardiovascular health protective effects as estrogen. Nevertheless, results from studies on the effect of physical activity on vascular function and cardiovascular health are inconsistent, with some studies showing a lack of effect of a physical activity program and others showing a beneficial effect. The reason for this divergence is unclear but here we explore whether there may be a timing aspect also for exercise training, *the exercise timing hypothesis,* where initiation of exercise interventions soon after menopause may be more effective than initiation many years after. The possibility that estrogen related receptor-α and oxidative stress may play a role for such a timing effect are discussed.

This is an Accepted Article that has been peer-reviewed and approved for publication in the The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/JP277056.

Introduction

The onset of menopause, with a consequent loss of estrogen, is associated with an increased risk of cardiovascular events, in part due to impaired vascular endothelial function, a rise in blood pressure and an increased risk of atherosclerosis (Mozaffarian *et al.*, 2015). Hormone replacement therapy was initially thought to counteract the decline in cardiovascular health in women after menopause (Grady *et al.*, 1992) but studies such as The Women's Health initiative reported an increased risk of cardiovascular events in women receiving hormone treatment, with the risk being most pronounced in women who were many years beyond menopause (Naftolin *et al.*, 2004; Rossow *et al.*, 2010). More recent studies; The Kronos Early Estrogen and Prevention Study (KEEPS) (Miller *et al.*, 2009) and The Early Versus Late Intervention Trial (ELITE) (Hodis *et al.*, 2016) have shown that hormone therapy may have beneficial effects on cardiovascular health if treatment is begun within the first six years of menopause but not in late menopausal women. This time dependent effect of treatment is referred to as "*The timing hypothesis"*. The mechanism underlying the timing hypothesis has not yet been fully elucidated.

The main question we raise in the current symposium review is whether there may be a similar aspect of timing of exercise training after menopause; "*The exercise timing hypothesis"*. Regular exercise training is clearly beneficial for men at all ages and numerous studies have shown that men up to very old age profit from training in terms of vascular and cardiac function (Schmidt *et al.*, 2014; Vorup *et al.*, 2017). In women at older age however, there is limited and conflicting data on a positive effect of exercise training on cardiac (Spina *et al.*, 1993; Egelund *et al.*, 2017) and vascular function (Pierce *et al.*, 2011*a*; Moreau *et al.*, 2013; Nyberg *et al.*, 2016; Seidelin *et al.*, 2017). Here we propose that greater improvements in vascular function may be achieved in women if training is initiated at middle age, soon after menopause, as opposed to many years (5-10 years) after menopause. The rationale behind this thinking stems from observations in studies on women at or beyond middle age, where some studies show no improvement in cardiovascular function after a period of physical activity (Spina *et al.*, 1993; Pierce *et al.*, 2011*b*; Moreau *et al.*, 2013) whereas others show a substantial improvement (Nyberg *et al.*, 2014; 2016; Egelund *et al.*, 2017). One of the differences in design between these studies was the age of the postmenopausal women, where vascular benefits were achieved in recent (Nyberg *et al.*, 2014; 2016) but not late (Pierce *et al.*, 2011*a*; Moreau *et al.*, 2013) postmenopausal women. Moreover, published and unpublished data from our group indicates that the estrogen related receptor-α, as assessed in

whole muscle, may be involved in adaptations in the recent but potentially not in the late postmenopausal period (Nyberg *et al.*, 2016); Gliemann & Hellsten, unpublished observations). As discussed further below, it is worth noticing that evidence is limited to a few studies and that the subjects and the methodologies used differ between the studies. A third potential reason for a timing effect could be the same as indicated in studies on hormone replacement therapy (Hodis *et al.*, 2016), that prevention of a decline in cardiovascular health may be more easily achieved than reversal of already established detrimental changes in the vascular system. Below we will discuss the role of exercise training in attenuating the detrimental impact of estrogen loss, address the idea that timing of exercise training after menopause may be of importance and that estrogen related receptor-α and oxidative stress could be underlying mechanisms.

Effects of estrogen on vascular function

A thorough overview on the effect of estrogen on vascular endothelial function is provided by Novella et al. in this same symposia series (publication office; please insert ref once available) in this symposia review collection on *Vascular function, exercise and estrogen*, therefore, only a brief account of the estrogen pathways and effects is provided here.

Estrogen can exert both genomic and non-genomic effects in the cardiovascular system and contributes to the regulation of vascular tone, vascular remodeling and protection against atherosclerotic plaque buildup. Estrogen mediates its effects by acting on the estrogen receptors ERα, ERβ and G protein coupled estrogen receptor 1 (GPER1, figure 1). These receptors are located in skeletal muscle (Wiik *et al.*, 2009*a*) and in endothelial and smooth muscle cells of the vascular wall (Haas et al. 2007). The genomic effect of estrogen is mediated primarily by activation of intracellular ERα or ERβ leading to translocation to the cell nucleus where the estrogen-estrogen receptor complex acts as a transcription factor regulating gene expression. Part of this effect is via binding to the estrogen response element (ERE). Activation of ERα located at the plasma membrane, activates the phosphatidylinositol-3-OH kinase (PI3K) and mitogen activated protein kinase (MAPK) pathway leading to eNOS activation (Lantin-Hermoso *et al.*, 1997; Chen *et al.*, 1999; Simoncini *et al.*, 2000). In the cardiovascular system, ERα has been described to be the primary receptor and most studies on the role of estrogen in the cardiovascular system have examined the importance of ERα with less being known about the role of ERβ. The expression of ERα assessed in venous endothelial cells fluctuates with the estrogen levels

during the menstrual cycle and the level of expression in older postmenopausal women is similar to that of the low estrogen period (early follicular phase) of the menstrual cycle (Gavin *et al.*, 2009; Hellsten & Gliemann, 2018).

The estrogen receptor GPER, a G protein coupled receptor, is located at the cell-membrane and is primarily involved in the non-genomic pathway. Activation of GPER by estrogen leads to activation of cellular pathways including endothelial nitric oxide synthase (eNOS) and inhibition of smooth muscle cell proliferation (Li *et al.*, 2013). Studies have suggested that GPER is the main receptor responsible for the vasodilator effect of estrogen (Sudhir *et al.*, 1995; Shaw *et al.*, 2000) via eNOS activation (Meyer *et al.*, 2010) and via an endothelium independent pathway (Yu *et al.*, 2011). However, further work is required to determine the specific physiological roles of the different estrogen receptors.

The timing hypothesis and underlying mechanisms

After the initial studies showing a potential detrimental effect of hormone replacement therapy on cardiovascular health primarily in late postmenopausal women, studies were conducted to elucidate the influence of time after menopause. In the ELITE study, the effect of hormone replacement therapy was compared in women within 6 years after menopause with women treated after more than 10 years post menopause (Hodis *et al.*, 2016). The study showed that the progression of atherosclerotic plaque build-up was lower in the early compared to the late menopausal women (Hodis *et al.*, 2016). A similar finding was made in non-human primates subjected to experimentally induced menopause where a lack of effect of estrogen treatment was observed once atherosclerotic plaques had been established (Mikkola & Clarkson, 2002). In fact, late treatment increased the risk of plaque rupture and thrombosis (Mikkola & Clarkson, 2002). Timing of estrogen treatment may also affect low-grade inflammation. Novella and co-workers cultured uterine arteries from postmenopausal women and found that incubation with estrogen induced a pro-inflammatory profile in the arteries from women who were more than five years after menopause, whereas in arteries from women closer to menopause, an anti-inflammatory effect was observed (Novella *et al.*, 2012).

In relation to the above, it is noteworthy that estrogen treatment has been shown to improve vascular function, assessed as brachial artery flow mediated dilation, in older post-

menopausal women (Moreau *et al.*, 2013). This discrepancy underlines the complexity of the role of estrogen in the cardiovascular system in women after menopause.

Mechanistic evidence explaining the importance of early versus late treatment is scarce but there is data to suggest that ERα density is lowered (Gavin *et al.*, 2009) and ERβ increased (Novella *et al.*, 2012) with age. ERα density has also been found to be higher in arteries with already established signs of atherosclerosis (Losordo *et al.*, 1994) and, in addition to changes in receptor expression, the function of ERα may be altered due to methylation of the promotor region (Post *et al.*, 1999). At the cellular level, such estrogen receptor changes may reduce or completely abolish the effects of estrogen on vascular health.

A parallel to the timing hypothesis of estrogen in the cardiovascular system is the observation that insulin stimulated glucose uptake into skeletal muscle is improved by estrogen therapy in early but not late postmenopausal women (Pereira *et al.*, 2015). On the mechanistic side, skeletal muscle of the late postmenopausal women had fewer ERα and ERβ receptors than recent postmenopausal women and estrogen treatment was found to increase PGC-1α and AMPK expression only in the recent postmenopausal women (Park *et al.*, 2017). Such data further support a role of alterations in estrogen receptor distribution and/or function in the timing aspect.

With regards to the timing of exercise training, it is plausible that alterations in estrogen levels and estrogen receptors with time after menopause influence the effect of exercise in postmenopausal women. However, timing may also be related to a secondary effect, where detrimental changes in atherosclerosis and vascular function due to the loss of estrogen reduce the efficacy of training. There is evidence to suggest that it is more difficult to achieve reversal of already established cardiometabolic diseases than to prevent or delay early changes. The age-associated changes in low grade inflammation and increased oxidative stress in the cardiovascular system are likely to counteract exercise training induced improvements in the antithrombotic and vasodilatory effects of NO. In fact, the finding that estrogen in post-menopausal women improves flow mediated dilation and allows for improvements with exercise training has been suggested to be related to an estrogen induced reduction in oxidative stress (Moreau *et al.*, 2013).

Nevertheless, more direct effects of estrogen receptors should be considered. In a study on muscle cell cultures it was observed that muscle contraction, in the absence of estrogen, could activate the estrogen response element (Wiik *et al.*, 2009*b*), an effect which may have been

due to activation of one of the estrogen receptors, or of the estrogen related receptor-α. Thus, it is plausible that muscle contraction directly can induce similar effects as estrogen. The finding that estrogen is required for training effects in older postmenopausal women but not in younger, could also suggest a direct coupling between muscle contraction and the estrogen receptors but the mechanism of such a coupling is unclear. These aspects are further discussed in the sections below.

Effects of exercise training on vascular function in mid-life women

It is well established that physical activity and exercise training improves cardiovascular function in men and cross sectional data shows that vascular function is better in life-long physically active older men compared to sedentary older men and that vascular function of active older men are comparable to that of young healthy men (Pierce *et al.*, 2011*b*; Nyberg *et al.*, 2012; Green *et al.*, 2017).

In contrast to the relatively well documented effect of exercise training in men, there is a paucity of studies in the literature related to cardiovascular function in women. Please see the thorough overview provided by Seals et al. from the same symposia series (publication office; please insert ref once available). Only a few studies have been published with data on how vascular function develops as function of age in women and the effects of exercise training on vascular function in women is controversial. It has been shown that flow mediated dilation is improved after a period of exercise training in aged men but not in age-matched post-menopausal women (Pierce *et al.*, 2011*a*) and a cross sectional study reported that life-long trained post-menopausal women express a similar vascular dysfunction as their sedentary counterparts (Santos-Parker *et al.*, 2017). The explanation for these observations remain largely unknown but attempts have been made to elucidate the underlying mechanisms; when exercise training is combined with estradiol supplementation (oral or transdermal), post-menopausal women are able to improve their vascular function to the same extent as age-matched men and pre-menopausal women (Moreau *et al.*, 2013). In addition, positive effects of exercise training in post-menopausal women are apparent when free radicals are scavenged by infusion of ascorbic acid suggesting that the underlying mechanism of the combined effect of estrogen and exercise training are associated with the antioxidant properties of estrogen (Moreau *et al.*, 2013). Supplementation of tetrahydrobiopterin (BH4), an essential co-factor for eNOS activity, rescues vascular function in post-menopausal women (Moreau *et al.*, 2012) and collectively, this suggest that mechanisms related to NO

bioavailability are key for the response to exercise training in post-menopausal women. However, further evidence for the cellular mechanism behind the apparent lack of effect of exercise training on vascular function after the menopausal transition is warranted. Contrary to these findings, recent studies have shown that vascular function is improved in both late pre- and recent post-menopausal women after a period of intense exercise training when vascular function is tested by femoral arterial infusion of acetylcholine and epoprostenol (Nyberg *et al.*, 2016). Circulating markers of systemic inflammation and atherosclerosis; the soluble intercellular adhesion molecule-1 and soluble vascular adhesion molecule-1, have also been shown to be reduced to the same extent in pre- and recent post-menopausal women (Nyberg *et al.*, 2014). In addition, training-induced adaptations in cardiac function were similar in pre- and recent post-menopausal women and exercise training lowered platelet reactivity in both groups (Egelund *et al.*, 2017; Lundberg Slingsby *et al.*, 2017). Collectively, these findings indicate that the trainability of the cardiovascular system is preserved in the early postmenopausal phase and combined with the observations that life-long trained post-menopausal women may or may not have better vascular function than their sedentary counterparts (see figure 2) indicate the importance of future studies that clearly show when or if the trainability diminishes after menopause.

The idea of a timing hypothesis for exercise training in postmenopausal women

In this brief review we bring forth the *exercise timing hypothesis* related to when exercise is initiated after menopause. This hypothesis is similar to the previously discussed timing hypothesis on estradiol supplementation and suggests that the positive effects of exercise are only present if regular physical activity is initiated in the early post-menopausal phase. The exercise timing hypothesis is based on the combined observations that vascular function in late post-menopausal women is not improved after a period of exercise training (Moreau *et al.*, 2013), that habitually active post-menopausal women have the same impaired vascular function as their sedentary counterparts (Santos-Parker *et al.*, 2017) while vascular function is improved to the same extent, or even better, in recent-menopausal compared to late pre-menopausal women (Nyberg *et al.*, 2014; 2016).

Thus, it is suggested that exercise training is more effective in improving vascular function in the early post-menopausal phase and the effects of exercise training will either decrease gradually as function of years after menopause or cease more abruptly at a certain, but yet

unknown menopausal age. Below we discuss mechanisms which could be the underlying cause of this phenomenon.

Estrogen related receptor-α as key element for training adaptations

From a molecular perspective, one explanation for the difference in response to exercise training in early- and late-postmenopausal women reside in the molecular signals that are initiated by either muscle activity or estrogen. As described in detail above, estrogen acts by binding to the estrogen receptors that subsequently cause transcriptional activation via the estrogen response element (Ciana *et al.*, 2003). Estrogen related receptors are orphan nuclear receptors similar to estrogen receptors, but which do not bind estrogen (Giguère *et al.*, 1988). Instead, estrogen related receptors are activated by a mitogen-activated protein kinase (MAPK) and peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) dependent pathway (Ariazi *et al.*, 2007; Wiik *et al.*, 2009*b*) and, similarly to the estrogen receptors, estrogen related receptors are able to cause transcriptional activation via estrogen response element (Ciana *et al.*, 2003). Exercise training also activates the MAPK and PGC1α pathway and muscle contraction of cultured skeletal muscle cells have been shown to activate estrogen response element via a MAPK dependent pathway (Wiik *et al.*, 2009*b*). Moreover, estrogen related receptors have been shown to regulate mitochondrial biogenesis and to be essential for exercise tolerance and muscle fitness in animal models (Schreiber *et al.*, 2004; Perry *et al.*, 2014). In humans, acute exercise increases mRNA levels of estrogen related receptors in the skeletal muscle (Cartoni *et al.*, 2005). Collectively, both estrogen and exercise can activate pathways to promote gene transcription and non-genomic activation of enzymes involved in vascular function. This includes upregulation and activation of eNOS but also upregulation of the mitochondrial antioxidant superoxide dismutase 2 (Huang *et al.*, 1997; Sumi & Ignarro, 2003). Since NO is an essential molecule for maintaining optimal vascular function, both the estrogenic and non-estrogenic (i.e. exercise) pathway is likely to be involved in governing vascular function in women (Craige *et al.*, 2016).

Until the menopausal transition, estrogen response element can be activated both by estrogen via estrogen receptors (Ciana *et al.*, 2003) and by exercise, potentially via estrogen related receptor-α (Wiik *et al.*, 2009*b*) and these pathways, with their link to NO bioavailability (Huang *et al.*, 1997; Sumi & Ignarro, 2003), are likely central mechanisms underlying the protective effects of estrogen and exercise on the cardiovascular system (figure 1). The important point here is that, after the cessation of estrogen production with menopause,

estrogen response element can still be activated through muscle activity leading to changes such as increased eNOS expression and activation, via the estrogen related receptor-α pathway. This clearly emphasizes the relevance of exercise training at menopause for maintaining or improving vascular function. In support of this theory, it has been shown that a period of exercise training increases the expression of estrogen related receptor-α in recent post-menopausal women, but not in pre-menopausal women, in parallel with a greater increase in the eNOS expression in the recent post-menopausal women, suggesting that activation of the estrogen related receptor-α pathway is of greater importance for the training adaptations in the recent postmenopausal group and that the presence of estrogen in the premenopausal group may limit the estrogen related receptor-α pathway (Nyberg *et al.*, 2017). Unpublished observations from our lab also indicate that the expression of estrogen related receptor-α is reduced as function of years after menopause in sedentary women (see figure 3). This could potentially explain the finding that late-postmenopausal women are unable to improve their vascular function by exercise training (Moreau *et al.*, 2013). Exploratory analysis from the Moreau *et al*. 2013 study indicated that improvements in endothelial function after exercise training was observed only in prior hormone replacement therapy users but not in the post-menopausal women who had never used hormone replacement therapy which could support that the duration where the estrogen response element is not activated via estrogen receptors is critical for the outcome of a training intervention (Moreau *et al.*, 2013). On this basis, we propose that, although it is never too late to begin training, it may be most beneficial for women to begin exercise training around the menopausal transition.

Age related vascular impairment- the hard way back

Another aspect of the observation that exercise training does not improve vascular function in late-postmenopausal women is that impairments of the vasculature beyond a point, particularly of the endothelial cells, becomes progressively difficult to reverse (Francis & Pierce, 2011). In line with this, it has been shown that the progression of atherosclerosis after menopause can be offset in early, but not late, post-menopausal women by oral estradiol supplementation (Hodis *et al.*, 2016). Atherosclerosis is initiated by the response of the endothelial cells to insult caused by a prolonged period of suboptimal conditions which may arise from lifestyle factors including hypertension, hyperlipidemia, hyperglycemia, free radicals and abnormal shear stress (Lusis, 2000). The pathogenesis of atherosclerosis is

multifactorial and progresses from functional to structural alterations. For detailed review see (Francis & Pierce, 2011). In short, initial endothelial injury impairs endothelial function and causes increased endothelial permeability that allows for accumulation of low-density lipoproteins within the tunica intima of the arterial vessel wall. The endothelial cell then releases inflammatory, aggregatory and adhesion molecules. Leukocytes are infiltrated in the vessel wall and the formation of a fibrous cap and smooth muscle cell migration and proliferation occurs when atherosclerosis is most progressed. The reversal of atherosclerosis becomes a particular difficult process once structural changes has occurred, and this could very well coincide by the time early menopause translate into late menopause. The reversal includes removal of lipids and necrotic material, regression of smooth muscle cells and restoration of endothelial and smooth muscle function (Francis & Pierce, 2011). Collectively, once manifested, reversal of more severe atherosclerosis requires substantial time and is not easily achieved and therefore promoting delay of atherosclerosis and plaque build-up by initiation of physical activity earlier on in life is likely to be beneficial. It should, nevertheless, be pointed out that other lifestyle factors as well as genetics influence atherosclerosis thus not all postmenopausal women will show severe atherosclerosis even with a sedentary life-style.

Imbalance between formation and removal of reactive oxygen species with advancing age and exercise

The production of reactive oxygen species (ROS) increases with advancing age while the endogenous antioxidant system is reduced, collectively leading to increased oxidative stress (Stadtman, 2001). The effects of ROS on substances important for optimal vascular function are multifactorial (Assar *et al.*, 2013)). First, ROS readily scavenge NO by reacting with NO to form peroxynitrite (Schulz *et al.*, 2008; Seals *et al.*, 2011). Secondly, ROS cause uncoupling of the homodimeric enzyme eNOS resulting in production of superoxide at the expense of NO production (Higashi *et al.*, 2002; Förstermann, 2006). Thirdly, free radicals readily inactivate prostacyclin synthase whereby the substrate for prostacyclin formation is shifted toward synthesis of the vasoconstrictor thromboxane via thromboxane synthase (Gluais *et al.*, 2005). A role for estrogen in oxidative stress has been demonstrated by infusion of either the antioxidant ascorbic acid or the prostanoid inhibitor indomethacin restoring acetylcholine induced vasodilation in ovariectomized women indicating that both free radicals and prostanoid derived vasoconstrictors are involved in the impaired vascular function caused by estrogen deprivation (Virdis *et al.*, 2000). Since menopause is also

associated with a reduction of the endogenous antioxidant enzymes in both endothelial cells and erythrocytes (Bellanti *et al.*, 2013; Liu *et al.*, 2014), this could in theory counteract any exercise training induced improvement of the enzymatic production of vasodilators.

Exercise increases the formation of ROS in skeletal muscles which are likely to be important signals mediating the training effect (Davies *et al.*, 1982; Silveira *et al.*, 2003; Hellsten *et al.*, 2007) and in a healthy system, excessive ROS formation during exercise is balanced by endogenous antioxidants in the skeletal muscle cells and the vascular wall. However, if the endogenous antioxidant defense is reduced, as observed with both aging and menopause (Bellanti *et al.*, 2013; Liu *et al.*, 2014), it is likely that the formation of ROS is inadequately matched by antioxidants and the ROS levels may become detrimental. The endogenous antioxidant system has been shown to be increased after a period of exercise training in both young and aged men (Gliemann *et al.*, 2013; 2014) but the effects of exercise training on endogenous antioxidants across the menopausal transition is unknown. Together, it could be speculated that exercise training should be initiated before the endogenous antioxidants starts to decline as a consequence of estrogen deprivation in order to enhance the antioxidant system before the oxidative stress becomes too large.

Summary and important considerations

In summary, the underlying mechanism that give raise to *the exercise timing hypothesis* may reside in i) a gradual decline in expression of estrogen related receptor-α or other molecular changes causing the exercise signal to be lost. ii) a functional to structural progression of atherosclerosis which becomes difficult to reverse and/or iii) an impaired antioxidant defense system that causes exercise derived ROS to be less of an exercise signal molecule and more a detrimental oxidative stressor. The estrogen related receptor-α theory is interesting and potentially important and should be further explored. However, in general, further studies including both cell, animal and human models are clearly needed to confirm existence of the exercise timing hypothesis and the underlying mechanisms.

It is important to note that not all observations in the literature support the concept of *the exercise timing hypothesis*. As such, Pierce *and co-workers* (Pierce *et al.*, 2011*b*) reported that there was no effect of aerobic exercise training on brachial artery flow mediated dilation at any time after menopause. Moreover, data on lifelong trained late menopausal women indicate that forearm vascular function is similar to that of age-matched

sedentary women (Pierce *et al.*, 2011*b*; Santos-Parker *et al.*, 2017). Leg vascular function, on the other hand, as assessed by femoral arterial infusion of acetylcholine, is better in lifelong trained late post-menopausal women compared to sedentary age-matched women (L. Gliemann & Y. Hellsten, unpublished findings, fig. 2). Yet, similar to our previous finding in men (Nyberg *et al.* 2014), leg vascular function in the life-long trained women is impaired compared to that of younger women pointing at a non-trainable age-induced impairment in both men and women (fig. 2). Therefore, the sex-specific lack of vascular improvement in life-long trained women observed by Santos Parker et al. (Santos-Parker *et al.*, 2017) may primarily be related to the remote effect of leg training on the arm vasculature. As previous studies have suggested that the effect of leg training on brachial artery flow mediated dilation is associated with shear stress (Birk *et al.* 2012) one underlying mechanism could be that shear stress sensing is impaired in postmenopausal women.

Evidence for an effect of exercise training, or lack her of, on cardiovascular health in women is still limited. Only a few laboratories have published on this important aspect of normal human aging and it should be emphasized that the methodologies used for evaluating vascular function in post-menopausal women differ between studies. As an example of a pivotal difference, our laboratory measures vascular function in the leg and uses intra-arterial infusion of vasoactive compounds while other laboratories use the arm as the experimental limb and use the non-invasive flow mediated dilation technique to evaluate vascular function. Such discrepancies may explain some of the differences in findings between studies. One aspect also worth emphasizing is that improvements after exercise training are generally observed in studies where vascular function is assessed in the leg which most likely reflects that improvements are most prominent in the trained limbs.

If the exercise timing hypothesis holds true, it will provide a basis for important recommendations to women with regard to the beneficial effects of physical activity through the ages. The menopausal transition can have a severe impact on health status and wellbeing in general, and women will benefit from clear recommendations on whether there may be an advantage to initiate a physically active life-style before or at menopause rather than later on in life. However, it should be strongly emphasized that this does not mean that it is too late to begin being more physically active later in life, physical activity is beneficial at all ages. Likewise, although significant changes in vascular function may be difficult to achieve in late post-menopause, exercise training still provides a plethora of health benefits.

Additional information

The authors report no conflict of interest, contributed equally to the manuscript and approved the final version of the manuscript. Both authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Figures and legends

Figure 1. Activation of estrogen response element (ERE) and eNOS by estrogen (β17 estradiol) and muscle contraction. ERE can be activated by muscle contraction via the MAPK - PGC1α - estrogen related receptor-α (ERRα) pathway. This pathway leads to genomic upregulation of endothelial nitric oxide synthase (eNOS) which may improve regulation of vascular function by increasing NO bioavailability. Estrogens activates ERE directly via estrogen receptors (ER) activation and may also lead to improved vascular regulation via increased eNOS expression and activate eNOS by phosphorylation of residue serine^{1177} via Phosphoinositide 3-kinase (PI3K) – Protein kinase B (Akt) pathway. Both exercise and estrogen also phosphorylate eNOS via PGC1α and G-protein coupled estrogen receptor 1 (GPER1), respectively. Collectively, both estrogen and exercise can reduce plaque formation and improve vascular function and arterial remodeling. The interaction between estrogens and exercise, in the pre-, recent post- and late post-menopausal phase is not fully understood.

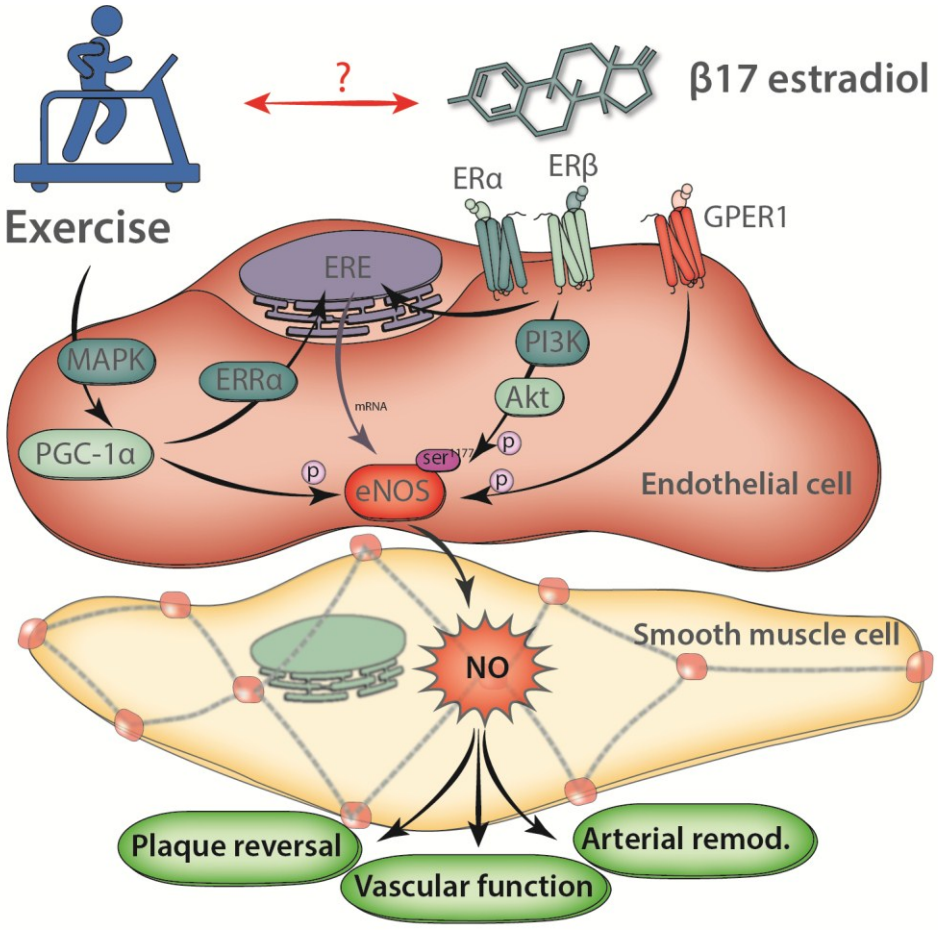


Figure 2. Changes in vascular conductance induced by femoral arterial acetylcholine infusion (100 μg · min^{-1} · L leg $volume^{-1}$) in sedentary pre-menopausal (n=20), recent post-menopausal women (n=16, 4 years since last menstruation), late post-menopausal women (n=11, 9 years since last menstruation and n=10, 17 years since last menstruation) and lifelong trained post-menopausal women (n=12, 14 years since last menstruation). Age (years) and fitness level (VO_{2max}; ml O_2 · kg^{-1} · min^{-1}) are presented in the upper box. Data are mean ± SE and combined from Nyberg et al 2016 and unpublished observations. The statistical analysis was run R-studio with a linear mixed model. * lower than **pre meno**; # lower than **3y post**; § higher than **9y and 17y post**. Note the marked decrease in vascular response to acetylcholine infusion at 9y and 17y post menopause and that this decline is attenuated to some extent, but not fully, in the lifelong trained group.

Age	50	54	59	66	65
VO_{2max}	30.6	31.9	27.1	27.4	41.5

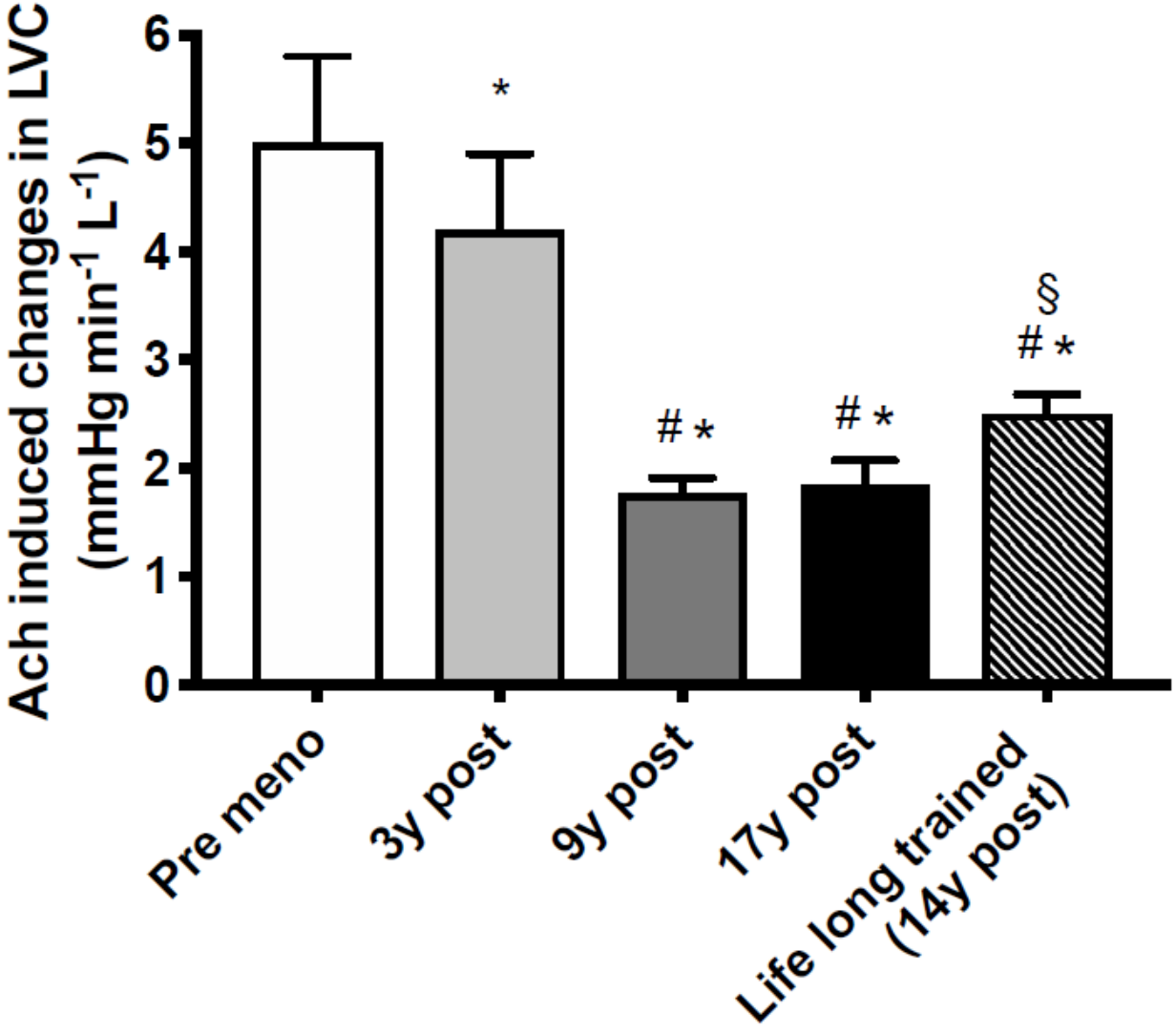
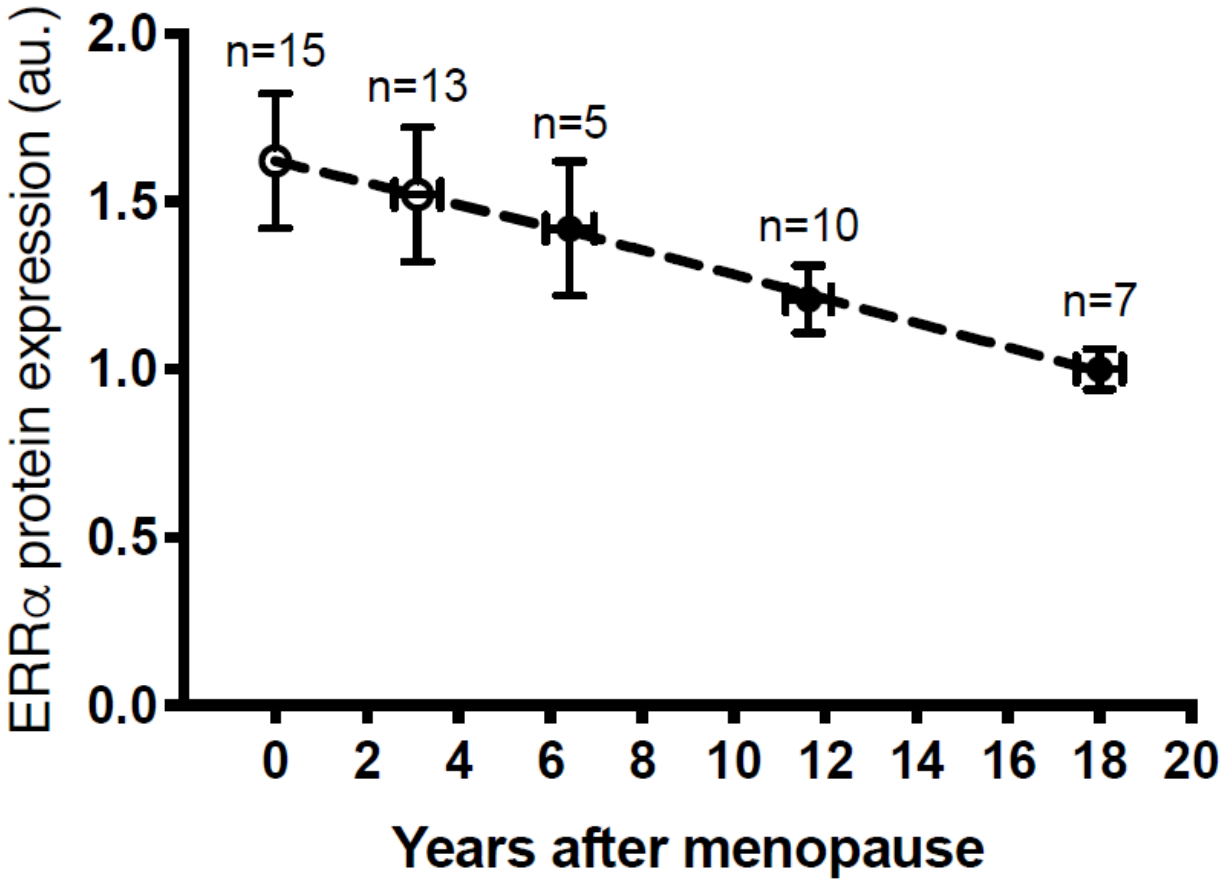
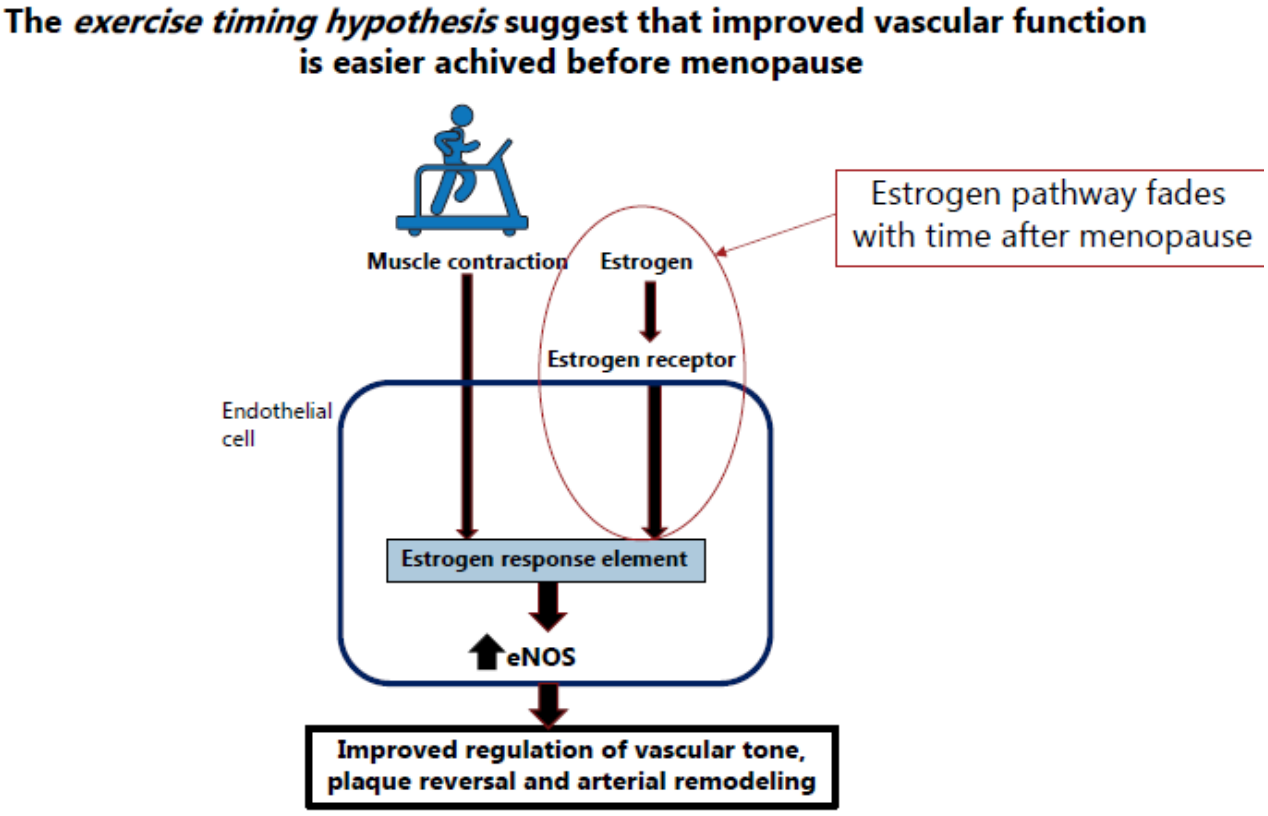


Figure 3. Protein expression of estrogen related receptor α (ERRα) as function of time after menopause in skeletal muscle homogenates from sedentary pre-menopausal and post-menopausal women. Data are mean ± SE and combined from Nyberg et al 2016 (open circles) and unpublished observations (closed circles).



Abstract figure. Exercise training initiates cellular signals that includes activation of estrogen response element which leads to increased expression and activation of endothelial nitric oxide synthase (eNOS). This improves vascular tone, increases plaque removal and arterial remodeling resulting in improved vascular function. Estrogen induces similar improvements to the vascular system and the exercise timing hypothesis suggest that exercise induced improvements to the vasculature is easier achieved before the menopausal transition when both estrogen and exercise can activate these pathways compared to after menopause.



References

Ariazi EA, Kraus RJ, Farrell ML, Jordan VC & Mertz JE (2007). Estrogen-related receptor alpha1 transcriptional activities are regulated in part via the ErbB2/HER2 signaling pathway. *Mol Cancer Res* **5,** 71–85.

Assar El M, Angulo J & Rodríguez-Mañas L (2013). Oxidative stress and vascular inflammation in aging. *Free Radical Biology and Medicine* **65,** 380–401.

Bellanti F, Matteo M, Rollo T, De Rosario F, Greco P, Vendemiale G & Serviddio G (2013). Sex hormones modulate circulating antioxidant enzymes: impact of estrogen therapy. *Elsevier* **1,** 340–346.

Birk GK, Dawson EA, Atkinson C, Haynes A, Cable NT, **Thijssen** DH & Green DJ (2012). *J Appl Physiol (1985)*. 112(10):1653-8.

Cartoni R, Léger B, Hock MB, Praz M, Crettenand A, Pich S, Ziltener J-L, Luthi F, Dériaz O, Zorzano A, Gobelet C, Kralli A & Russell AP (2005). Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise. *J Physiol* **567,** 349–358.

Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME & Shaul PW (1999). Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin Invest* **103,** 401–406.

Ciana P, Raviscioni M, Mussi P, Vegeto E, Que I, Parker MG, Lowik C & Maggi A (2003). In vivo imaging of transcriptionally active estrogen receptors. *Nat Med* **9,** 82–86.

Craige SM, Kröller-Schön S, Li C, Kant S, Cai S, Chen K, Contractor MM, Pei Y, Schulz E & Keaney JF (2016). PGC-1α dictates endothelial function through regulation of eNOS expression. *Sci Rep* **6,** 38210.

Davies KJ, Quintanilha AT, Brooks GA & Packer L (1982). Free radicals and tissue damage produced by exercise. *Biochemical and Biophysical Research Communications* **107,** 1198–1205.

Egelund J, Jørgensen PG, Mandrup CM, Fritz-Hansen T, Stallknecht B, Bangsbo J, Nyberg M & Hellsten Y (2017). Cardiac Adaptations to High-Intensity Aerobic Training in Premenopausal and Recent Postmenopausal Women: The Copenhagen Women Study. *Journal of the American Heart Association* **6,** 233.

Förstermann U (2006). Janus-faced role of endothelial NO synthase in vascular disease: uncoupling of oxygen reduction from NO synthesis and its pharmacological reversal. *Biol Chem* **387,** 1521–1533.

Francis AA & Pierce GN (2011). An integrated approach for the mechanisms responsible for atherosclerotic plaque regression. *Exp Clin Cardiol* **16,** 77–86.

Gavin KM, Seals DR, Silver AE & Moreau KL (2009). Vascular endothelial estrogen receptor alpha is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women. *J Clin Endocrinol Metab* **94,** 3513–3520.

Giguère V, YANG N, SEGUI P & EVANS RM (1988). Identification of a New Class of Steroid-Hormone Receptors. *Nature* **331,** 91–94.

Gliemann L, Nyberg M & Hellsten Y (2014). Nitric oxide and reactive oxygen species in limb vascular function: what is the effect of physical activity? *Free Radic Res* **48,** 71–83.

Gliemann L, Schmidt JF, Olesen J, Biensø RS, Peronard SL, Grandjean SU, Mortensen SP, Nyberg M, Bangsbo J, Pilegaard H & Hellsten Y (2013). Resveratrol Blunts the Positive Effects of Exercise Training on Cardiovascular Health in Aged Men. *J Physiol* **591,** 5047–5059.

Gluais P, Lonchampt M, Morrow JD, Vanhoutte PM & Félétou M (2005). Acetylcholine-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. *British Journal of Pharmacology* **146,** 834–845.

Grady D, Rubin SM, Petitti DB, Fox CS, Black D, Ettinger B, Ernster VL & Cummings SR (1992). Hormone therapy to prevent disease and prolong life in postmenopausal women. *Ann Intern Med* **117,** 1016–1037.

Hellsten Y & Gliemann L (2018). Limb vascular function in women-Effects of female sex hormones and physical activity. *Translational Sports Medicine* **1,** 14–24.

Hellsten Y, Nielsen JJ, Lykkesfeldt J, Bruhn M, Silveira L, Pilegaard H & Bangsbo J (2007). Antioxidant supplementation enhances the exercise-induced increase in mitochondrial uncoupling protein 3 and endothelial nitric oxide synthase mRNA content in human skeletal muscle. *Free Radical Biology and Medicine* **43,** 353–361.

Higashi Y, Sasaki S, Nakagawa K, Fukuda Y, Matsuura H, Oshima T & Chayama K (2002). Tetrahydrobiopterin enhances forearm vascular response to acetylcholine in both normotensive and hypertensive individuals. *Am J Hypertens* **15,** 326–332.

Hodis HN, Mack WJ, Henderson VW, Shoupe D, Budoff MJ, Hwang-Levine J, Li Y, Feng M, Dustin L, Kono N, Stanczyk FZ, Selzer RH, Azen SPELITE Research Group (2016). Vascular Effects of Early versus Late Postmenopausal Treatment with Estradiol. *N Engl J Med* **374,** 1221–1231.

Huang A, Sun D, Kaley G & Koller A (1997). Estrogen maintains nitric oxide synthesis in arterioles of female hypertensive rats. *Hypertension* **29,** 1351–1356.

Lantin-Hermoso RL, Rosenfeld CR, Yuhanna IS, German Z, Chen Z & Shaul PW (1997). Estrogen acutely stimulates nitric oxide synthase activity in fetal pulmonary artery endothelium. *Am J Physiol* **273,** L119–L126.

Li F, Yu X, Szynkarski CK, Meng C, Zhou B, Barhoumi R, White RE, Heaps CL, Stallone JN & Han G (2013). Activation of GPER Induces Differentiation and Inhibition of Coronary Artery Smooth Muscle Cell Proliferation. ed. Kirchmair R. *PLoS ONE* **8,** e64771.

Liu Z, Gou Y, Zhang H, Zuo H, Zhang H, Liu Z & Yao D (2014). Estradiol improves cardiovascular function through up-regulation of SOD2 on vascular wall. *Elsevier* **3,** 88–99.

Losordo DW, Kearney M, Kim EA, Jekanowski J & Isner JM (1994). Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. *Circulation* **89,** 1501–1510.

Lundberg Slingsby MH, Nyberg M, Egelund J, Mandrup CM, Frikke-Schmidt R, Kirkby NS & Hellsten Y (2017). Aerobic exercise training lowers platelet reactivity and improves platelet sensitivity to prostacyclin in pre- and postmenopausal women. *J Thromb Haemost* **15,** 2419–2431.

Lusis AJ (2000). Atherosclerosis. *Nature* **407,** 233–241.

Meyer MR, Baretella O, Prossnitz ER & Barton M (2010). Dilation of epicardial coronary arteries by the G protein-coupled estrogen receptor agonists G-1 and ICI 182,780. *Pharmacology* **86,** 58–64.

Mikkola TS & Clarkson TB (2002). Estrogen replacement therapy, atherosclerosis, and vascular function. *Cardiovascular Research* **53,** 605–619.

Miller VM, Black DM, Brinton EA, Budoff MJ, Cedars MI, Hodis HN, Lobo RA, Manson JE, Merriam GR, Naftolin F, Santoro N, Taylor HS & Harman SM (2009). Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). *J Cardiovasc Transl Res* **2,** 228–239.

Moreau KL, Meditz A, Deane KD & Kohrt WM (2012). Tetrahydrobiopterin improves endothelial function and decreases arterial stiffness in estrogen-deficient postmenopausal women. *Am J Physiol Heart Circ Physiol* **302,** H1211–H1218.

Moreau KL, Stauffer BL, Kohrt WM & Seals DR (2013). Essential Role of Estrogen for Improvements in Vascular Endothelial Function With Endurance Exercise in Postmenopausal Women. *J Clin Endocrinol Metab* **98,** 4507–4515.

Mozaffarian D et al. (2015). Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* **131,** e29–e322.

Naftolin F, Taylor HS, Karas R, Brinton E, Newman I, Clarkson TB, Mendelsohn M, Lobo RA, Judelson DR, Nachtigall LE, Heward CB, Hecht H, Jaff MR, Harman SMWomen's Health Initiative (2004). The Women's Health Initiative could not have detected cardioprotective effects of starting hormone therapy during the menopausal transition. *Fertil Steril* **81,** 1498–1501.

Novella S, Heras M, Hermenegildo C & Dantas AP (2012). Effects of estrogen on vascular inflammation: a matter of timing. *Arterioscler Thromb Vasc Biol* **32,** 2035–2042.

Nyberg M, Egelund J, Mandrup CM, Andersen CB, Hansen KMBE, Hergel I-MF, Valbak-Andersen N, Frikke-Schmidt R, Stallknecht B, Bangsbo J & Hellsten Y (2017). Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. *J Physiol* **595,** 2969–2983.

Nyberg M, Egelund J, Mandrup CM, Nielsen MB, Mogensen AS, Stallknecht B, Bangsbo J & Hellsten Y (2016). Early Postmenopausal Phase Is Associated With Reduced Prostacyclin-Induced Vasodilation That Is Reversed by Exercise Training: The Copenhagen Women Study. *Hypertension* **68,** 1011–1020.

Nyberg M, Seidelin K, Rostgaard Andersen T, Neumann Overby N, Hellsten Y & Bangsbo J (2014). Biomarkers of vascular function in pre- and recent

post-menopausal women of similar age: effect of exercise training. *Am J Physiol Regul Integr Comp Physiol*; DOI: 10.1152/ajpregu.00539.2013.

Park Y-M, Pereira RI, Erickson CB, Swibas TA, Kang C & Van Pelt RE (2017). Time since menopause and skeletal muscle estrogen receptors, PGC-1α, and AMPK. *Menopause* **24,** 815–823.

Pereira RI, Casey BA, Swibas TA, Erickson CB, Wolfe P & Van Pelt RE (2015). Timing of Estradiol Treatment After Menopause May Determine Benefit or Harm to Insulin Action. *J Clin Endocrinol Metab* **100,** 4456–4462.

Perry M-C, Dufour CR, Tam IS, B'chir W & Giguère V (2014). Estrogen-related receptor-α coordinates transcriptional programs essential for exercise tolerance and muscle fitness. *Mol Endocrinol* **28,** 2060–2071.

Pierce GL, Donato AJ, LaRocca TJ, Eskurza I, Silver AE & Seals DR (2011*a*). Habitually exercising older men do not demonstrate age-associated vascular endothelial oxidative stress. *Aging Cell* **10,** 1032–1037.

Pierce GL, Eskurza I, Walker AE, Fay TN & Seals DR (2011*b*). Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults. *Clin Sci* **120,** 13–23.

Post WS, Goldschmidt-Clermont PJ, Wilhide CC, Heldman AW, Sussman MS, Ouyang P, Milliken EE & Issa JP (1999). Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. *Cardiovascular Research* **43,** 985–991.

Rossow L, Fahs CA, Guerra M, Jae SY, Heffernan KS & Fernhall B (2010). Acute effects of supramaximal exercise on carotid artery compliance and pulse pressure in young men and women. *Eur J Appl Physiol* **110,** 729–737.

Santos-Parker JR, Strahler TR, Vorwald VM, Pierce GL & Seals DR (2017). Habitual aerobic exercise does not protect against micro- or macrovascular endothelial dysfunction in healthy estrogen-deficient postmenopausal women. *J Appl Physiol* **122,** 11–19.

Schmidt JF, Hansen PR, Andersen TR, Andersen LJ, Hornstrup T, Krustrup P & Bangsbo J (2014). Cardiovascular adaptations to 4 and 12 months of football or strength training in 65- to 75-year-old untrained men. *Scand J Med Sci Sports* **24,** 86–97.

Schreiber SN, Emter R, Hock MB, Knutti D, Cardenas J, Podvinec M, Oakeley EJ & Kralli A (2004). The estrogen-related receptor alpha (ERRalpha)

functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis. *Proc Natl Acad Sci USA* **101,** 6472–6477.

Schulz E, Jansen T, Wenzel P, Daiber A & Münzel T (2008). Nitric Oxide, Tetrahydrobiopterin, Oxidative Stress, and Endothelial Dysfunction in Hypertension. *Antioxidants & Redox Signaling* **10,** 1115–1126.

Seals DR, Jablonski KL & Donato AJ (2011). Aging and vascular endothelial function in humans. *Clin Sci* **120,** 357–375.

Seidelin K, Nyberg M, Piil P, Jørgensen NR, Hellsten Y & Bangsbo J (2017). Adaptations with Intermittent Exercise Training in Post- and Premenopausal Women. *Med Sci Sports Exerc* **49,** 96–105.

Shaw L, Taggart MJ & Austin C (2000). Mechanisms of 17 beta-oestradiol induced vasodilatation in isolated pressurized rat small arteries. *British Journal of Pharmacology* **129,** 555–565.

Silveira LR, Pereira-Da-Silva L, Juel C & Hellsten Y (2003). Formation of hydrogen peroxide and nitric oxide in rat skeletal muscle cells during contractions. *Free Radical Biology and Medicine* **35,** 455–464.

Simoncini T, Hafezi-Moghadam A, Brazil DP, Ley K, Chin WW & Liao JK (2000). Interaction of oestrogen receptor with the regulatory subunit of phosphatidylinositol-3-OH kinase. *Nature* **407,** 538–541.

Spina RJ, Ogawa T, Kohrt WM, Martin WH, Holloszy JO & Ehsani AA (1993). Differences in cardiovascular adaptations to endurance exercise training between older men and women. *J Appl Physiol* **75,** 849–855.

Stadtman ER (2001). Protein oxidation in aging and age-related diseases. *Ann N Y Acad Sci* **928,** 22–38.

Sudhir K, Chou TM, Mullen WL, Hausmann D, Collins P, Yock PG & Chatterjee K (1995). Mechanisms of estrogen-induced vasodilation: in vivo studies in canine coronary conductance and resistance arteries. *Journal of the American College of Cardiology* **26,** 807–814.

Sumi D & Ignarro LJ (2003). Estrogen-related receptor alpha 1 up-regulates endothelial nitric oxide synthase expression. *Proc Natl Acad Sci USA* **100,** 14451–14456.

Virdis A, Ghiadoni L, Pinto S, Lombardo M, Petraglia F, Gennazzani A, Buralli S, Taddei S & Salvetti A (2000). Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. *Circulation* **101,** 2258–2263.

Vorup J, Pedersen MT, Brahe LK, Melcher PS, Alstrøm JM & Bangsbo J (2017). Effect of small-sided team sport training and protein intake on muscle mass, physical function and markers of health in older untrained adults: A randomized trial. ed. Calbet JAL. *PLoS ONE* **12,** e0186202.

Wiik A, Ekman M, Johansson O, Jansson E & Esbjörnsson M (2009*a*). Expression of both oestrogen receptor alpha and beta in human skeletal muscle tissue. *Histochem Cell Biol* **131,** 181–189.

Wiik A, Hellsten Y, Berthelson P, Lundholm L, Fischer H & Jansson E (2009*b*). Activation of estrogen response elements is mediated both via estrogen and muscle contractions in rat skeletal muscle myotubes. *Am J Physiol Cell Physiol* **296,** C215–C220.

Yu X, Ma H, Barman SA, Liu AT, Sellers M, Stallone JN, Prossnitz ER, White RE & Han G (2011). Activation of G protein-coupled estrogen receptor induces endothelium-independent relaxation of coronary artery smooth muscle. *Am J Physiol Endocrinol Metab* **301,** E882–E888.

